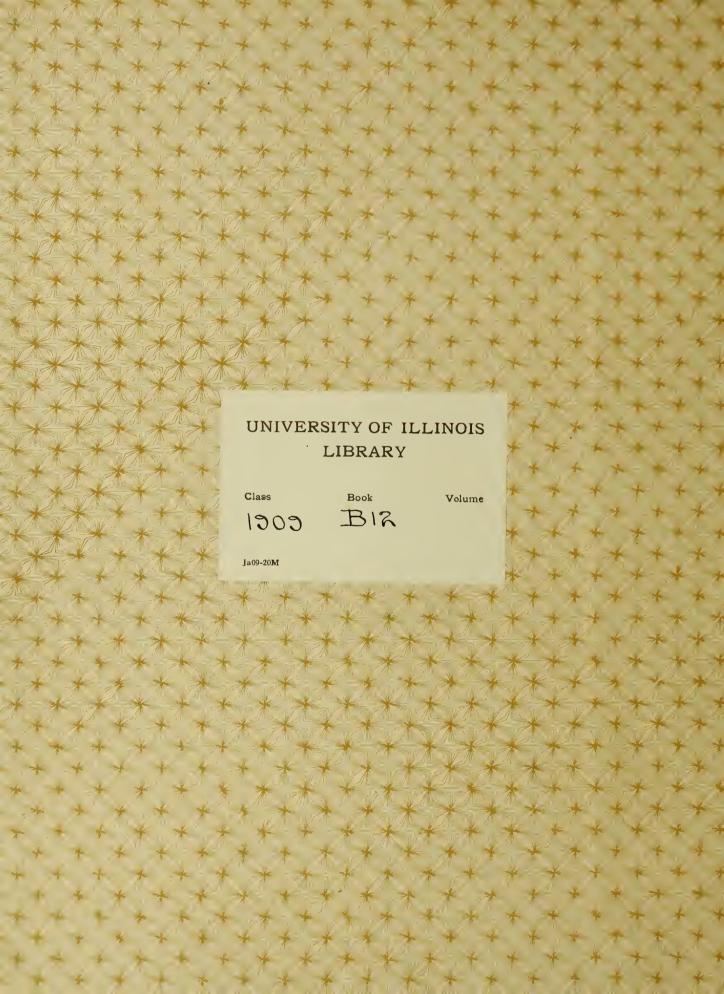
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A Study of the Histology of The Normal Adrenal Gland

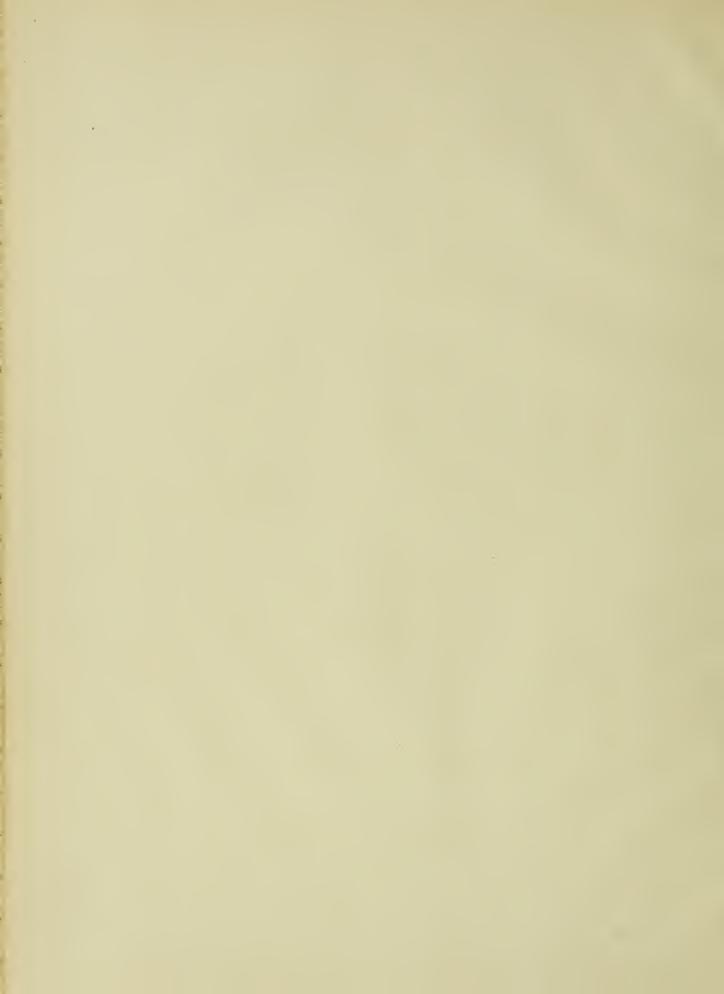
Physiology
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# A STUDY OF THE HISTOLOGY OF THE NORMAL ADRENAL GLAND



BY

#### IRWIN WOODWARD BACH

A.B. University of Illinois, 1908

#### THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF ARTS

IN PHYSIOLOGY

IN

THE GRADUATE SCHOOL

OF THE

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1909

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June 1

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Drwin Woodward Bach

ENTITLED Ce Study of the Histology of the Normal Edrewal Gland.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Master of Arts

John K. Molellan.
Head of Department

Recommendation concurred in:

Committee

on

Final Examination



Many investigations on the glands and glandular structures have proven that histological changes occur in the cells of
the gland, corresponding to different physiological phases of activity. That is to say, the histological appearance of the glands
during a period of rest differs from the appearance during a state
of active secretion. These changes have been observed both in fresh
and in preserved preparations.

Heidenhain in studying salivary glands hardened in alcohol noticed differences in glands taken from hungry or starved dogs, when compared with those taken from recently fed animals.

Langley confirmed the work of Haidenhain, in regard to the varying appearances of the cells in their different stages of activity, and brought out new points with reference to the subject. He found, that in the parotid gland during the quiescent stage, the alveolar cells were granular throughout. As the gland secreted, the granules disappeared from the outer borders of the alveolar cells, that is, the portion of the cell nearest the basement membrane; leaving very few granules in this region, after prolonged secretion. Those which remained assumed a definite form, arranging themselves in a thin layer at the portion of the cell bounding the lumen of the duct, and stretching outwards also as a thin layer "along the cell sides a variable distance from the lumen". These changes occurred whether the secretion was induced by feeding, by the use of pilocarpin, or by stimulation of the sympathetic nerve to the gland. Similar changes were observed by him in different degrees in the sub-maxillary, infra-orbital, lachrymal and certain mucous glands.

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Langley and Sewall found that the chief cells of the mammalian gastric gland were crowded with well defined granules during rest. During the process of digestion the granules in these chief cells diminished in number.

Kuhn and Lea working on rabbit's pancreas, noted that secretion was accompanied by vascular dilation, and they claimed to have seen granules of secretion carried from the base of the cell to the gland lumen.

A. Mathews in his study of the pancreas, found characteristic changes occurring in the cells of the gland as the result of feeding, or of stimulation of the vagus. In the resting gland, the granules differed in size and the smallest ones were usually found near the duct lumen. At the close of a long period of secretion, the cells showed a very large "outer zone", i.e. the part of the cell farthest from the lumen of the duct, which was free from granules, and a great amount of thread substance; while the granules disappeared almost entirely in the other part of the cell. The nucleus during this phase became large and round, and lay well towards the middle of the cell. Nearly the opposite conditions were seen at the close of long periods of rest. The "outer zone" had nearly or quite disappeared; the thread substance either was gone or lay at the back of the nucleus; and the granules were quite abundant. The nucleus had become probably a little smaller and might be slightly irregular in outline. Between these two extremes, all stages of transition could be observed. He found that changes of a similar nature took place in the submaxillary, orbital, sublingual, parotid, and sweat glands, in Bowman's glands, stomach glands, Brunner's glands, LieberKuhn's glands; and in the cells of

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the liver, either as the result of feeding, or of the use of pilocarpin, or by stimulation.

Not only have such histological changes been observed in glands whose secretions are carried away by ducts, but certain changes have also been observed in a few cases in glands with internal secretions, during activity.

Mathews also noticed in the thyreoid gland, that the cells degenerated in the periphery into colloid material; that all the protoplasm but the nucleus might be converted into colloid material; and that the nucleus always appeared in the "outer zone", that is, the portion of the cell nearest a blood vessel. He also mentioned relatively rare occurrences, in the pancreas, of certain bodies of yellowish color, like fat, which stained with osmium tetroxide. This he thought was the only histological evidence of an internal secretion in this gland. With these two exceptions, I find no record of glands having internal secretions, in which have been described histological changes due to their metabolic activities.

It was not until the work of Brown-Sequard in 1856, that the adrenal glands were supposed to have any specific function in the body. He found that the removal of these glands was followed by great prostration, muscular weakness, diminution in muscular tone and finally by death in from two to three days. His results proved conclusively that the adrenal glands were necessary for life. Later it was proven that the glands acted through an internal secretion. The evidences of the existance of such a secretion was, that on extracting the glands with glycerin, the extract had the power of raising the blood pressure when it was injected into another animal.

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This result was obtained from the extract of the medulla alone; cortical extract apparently produced no effect. From this it has been generally assumed that the medulla is the seat of formation of the active principle of the gland which produces the characteristic rise in blood pressure.

For the cortex, no function has been conclusively demonstrated though from evidence of a few pathological cases it has been suggested that it may exert some influence on the development of the sex glands.

Owing to the differences in the two parts of the gland, histologically, and probably in their embryological origin, a corresponding difference in function would seem not improbable. The proof then of the existence of an internal secretion from the gland is conclusive. The only evidence, however, of a nervous control of this secretion is based on the work of Dreyer who succeeded in producing an increase in the amount of secretion, by stimulating electrically the splanchnic nerve.

Biedl had previously attempted this but with negative results. Dreyer deduced physiological evidence as follows: various amounts of blood were taken during stimulation of the splanchnic nerve, by introducing a cannula into the adrenal vein. Other specimens were taken from the same source while the nerve was not being stimulated, and blood was also taken from the femoral vein in every case, before opening the abdomen, as a control. These samples were then defibrinated, filtered through muslin and injected into the jugular vein of another dog. The results of these injections was a rise in blood pressure in all cases, but a greater rise occurred when the blood taken from the stimulated gland was injected. This

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increase of the active principle in the blood is not referable to the effect on blood flow through the glands due to the stimulation of the vaso-dilators of the splanchnic. The increased secretion was observed whether the blood flow out of the vein increased, decreased, or remained constant, and therefore Dreyer concluded that secretory fibres ran to the adrenal in the splanchnic.

Jackson found that when adrenalin was injected into the blood of a dog, the active principle existed as such for about one minute's time, after which all traces of it were lost. He believed that this destruction of the adrenalin in the body was probably due to its oxidation. If the adrenalin is oxidized so quickly, it seems possible that the process of whipping the blood, which Dreyer used, would tend to produce the same results. In as much however, as Dreyer seems to have demonstrated clearly a difference in the character of the blood drawn under different conditions so far as the blood-pressure-raising principle is concerned, it is possible that there may be a special oxidative change of the active principle in the body.

It was with the hope of obtaining some histological verification of Dreyer's work that the work forming the subject of the present thesis was undertaken. Since characteristic changes occur in other glands as the result of stimulation, it seemed very probable that similar occurrences might be demonstrated in the adrenal.

#### METHODS.

The animals used in the experiment's were dogs, cats and guinea pigs. The stimulation experiments were performed entirely on dogs and cats. Ether was used in all cases as an anesthetic. The splanchnic nerve to the left adrenal was stimulated for different

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periods of time, ranging from ten minutes to two hours. The method of procedure of these experiments was varied in certain cases. In one animal the adrenal vein was prepared and occluded during stimulation so that if any granules were given off, they would remain in the venous opaces of the gland. The artery to the gland was occluded in another case, during a stimulation period of twenty minutes, with the purpose of more quickly exhausting the gland. At the end of stimulation, the gland was removed and portions were reserved for fresh examination, while the remainder was put into different fixing fluids. The right adrenal which had not been stimulated was also removed and treated in the same way as a control and comparative study. The fixing fluids used were Tellyesniczky's, Zenkers, Flemming's (strong) Formol-bichromate and 4% Formol. Acetic sublimate and alcoholic sublimate were tried but with unsatisfactory results in as much as they shrunk the cells and produced large vacuolated areas. The Zenker and Flemming solutions gave the best results. Sections were cut in paraffin 3 to 6 micra thick and the Zenker preparations were stained in Haidenhain's iron haemotoxylin, no contrast stain being used. No nuclear stain was used on the Flemming preparations as it was found that the study of the relations of the cell walls and nuclei to the granules was helped in no way thereby. Therefore the following method was employed. Tissues were fixed in strong Flemming in the dark for twenty four hours; washed in running water for one hour; placed in 1% pyrogallol for one hour; and then dehydrated embedded and sectioned as the other tissues. By this method, nuclei were impregnated and cell boundaries brought into view.

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#### The Chromaffine Reaction.

For some time it has been known that the medullary cells of the adrenal gland have a special affinity for, and give a characteristic reaction with chromic acid and its salts. chromaffine cells have been described in the carotid, and coccygeal glands as well. Schafer both demonstrated these and Stillung (10)cells in the carotid gland, and Kose described them in the carotid and adrenal glands of birds. The characteristic feature of these cells as implied in the name is their staining reaction when treated with Mullers fluid or some fluid containing chromic acid. assume under these conditions a deep brown color, in sharp contrast to the unstained cortical cells. By their intense staining with iron identified chromaffine cells in the medulla of haemotoxylin, Flint the adrenal gland fixed in Muller's solution and found when medullary cells were misplaced, as occasionally occurred, this was his surest way of identifying and distinguishing them from the surrounding cortical cells.

Schur and Wiesel stated that with any of the anesthetics after three quarters of an hour anesthesia there was a diminution in the number of patches of cells taking the chromaffine reaction in the medulla, and that this reduction was constant. After three to five hours there was no chromaffine reaction whatever, and extracts after five hours anesthesia gave no effect of mydriasis, would not raise blood pressure, nor give the iron chloride reaction. After probably twelve hours, the cells would regain their power to give this reaction again. From this they infer that the chromaffine reaction is in some way connected with the active principle of the

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gland, especially since it is only the extract of the medulla which has these powers. This paper was only a preliminary report, however, and few details of their work were given. If their results were correct it would be necessary in our work to find an anesthetic which would avoid these complications.

With these points in view, I started out then to verify their work and to find if possible an anesthetic which I might safely use, and still have a normal gland. These experiments were carried out on dogs, cats and guinea pigs. The following protocols show the range of the work. Other experiments were performed within the time limits of those given.

Dog No. 5. Weight 17 Kilograms.

9:00 A. M. Small amount of chloroform administered, then ether.

Tracheal cannula inserted by assistant. Abdomen opened.

9:26 A.M. Part of left adrenal gland removed and portions placed immediately in 7% saline solution at 37 C., and 4% formol. Other portions put into Tellyesniczky, Zenker, Flemming (strong), and formol-bichromate.

Abdomen sewed up and kept covered with hot towels.

Deep anesthesia maintained.

1:10 P.M. Abdomen opened for the second time and right adrenal removed and treated in the same was as the left adrenal.

1:18 P.M. Dog killed.

Guinea Pig No. 5.

1:45 P. M. Half grown guinea pig killed by a blow on head and both adrenals removed immediately. Portions were placed in 7% saline solution, 4% formol, Tellyesniczky, Zenker, Flemming (strong) and formol bichromate.

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Guinea Pig No. 2.

2:20 P.M. Nearly full grown guinea pig etherized

2:28 P.M. Abdomen opened and part of left adrenal removed. Portions were placed in 7% saline solution, 4% formol,
Tellyesniczky, Zenker, Flemming (strong) and formolbichromate. Abdomen was sewed up and kept covered with warm clothes.

Deep anesthesia maintained by dropping ether on cloth over animal's nose.

4:05 P.M. Abdomen re-opened and right adrenal taken out and treated in same way as the left gland.

Guinea Pig No. 4.

12:30 P. M. Half grown animal etherized.

12:40 P.M. Abdomen opened and part of left adrenal taken out. Portions were placed in 7% saline solution, 4% formol,

Tellyesniczky, Zenker, Flemming (strong) and formalbichromate.

Abdomen sewed up and kept covered with warm clothes.

Deep anesthesia maintained by dropping ether on cloth

over animal's nose.

5:40 P. M. Abdomen re-opened and right adrenal gland taken out and treated the same as the left gland.

The portions of the glands which were put up in the different fixing fluids were sectioned in paraffine. The remaining portions which were placed in the .7% saline solution, and in 4% formal were examined immediately after the following method. Free hand sections and frozen sections of each were made immediately, and treated with 3% potassium bichromate for 8 to 12 hours, and with 1% chromic acid for 6 to 8 hours. All paraffin sections were examined

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also by dissolving off the paraffin after the sections were on the slide and then mounting them in neutral balsam. The reaction showed certain differences according to the way in which the sections were treated. The free hand and frozen sections when treated with bichromate or chromic acid showed patches of medullary cells stained a deep brown color. These patches of cells appeared to have no definite arrangement in the medulla nor was there a constant number of cells in a patch. The reaction was unchanged by anesthesia. No difference was apparent, either in the relative number of patches taking the stain or in the intensity of their reaction, between the glands taken from the guinea pig which was knocked on the head and those from animals under anesthesia for five hours. Aside from this chromaffine reaction, these cells appeared identical with the other medullary cells. In no case did these patches of cells show in the paraffin sections, but when the sections were stained with iron haemotoxylin all medullary cells were stained darker than the cortical cells. This is the same reaction which Flint described. the other hand a chromaffine reaction did appear in the tissues treated with the formal-bichromate fixing fluid. In this case all medullary cells were stained evenly and uniformly a yellowish brown color. This is probably the same reaction as the patches of cells show, only in different degrees of intensity, and is due I think to the action of the formol in the capacity of an accentuator. This was unchanged also by anesthesia.

From the study of this chromaffine reaction, I think I am justified in drawing the following conclusions.

1.- The chromaffine reaction from the fixing agents, (formol bichromate excepted) if there be any, is destroyed by running

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the tissues up through the alcohols.

- This chromaffine reaction may be regained only in part by retreatment with formol-bichromate solution for twenty-four hours, but even this is very slight. 3% potassium bichromate or 1% chromic acid do not give this result in retreatment.

  Tissues put up in absolute alcohol (dog 6) however, do not give a chromaffine reaction even when treated with formol-bichromate solution.
- 3.- The reaction may be brought about and retained through washings, alcohols, and all subsequent treatment by using formol-bichromate as a fixing agent.
- 4.- Frozen sections or free hand sections made from tissue preserved in 4% formol, give the chromaffine reaction when treated with 3% pot. bichrom. or 1% chromic acid. This reaction however, is peculiar in that it does not extend over the whole medulla as the formol-bichromate reaction does, but gives the characteristic patches. These patches are not lost by treatment with the graded alcohols and subsequent procedure as they are in the other case mentioned. From these results it appears that formol is an accentuator to the chromium.
- 5.- Fresh tissues give the chromaffine reaction with 1% chromic acid and with 3% potassium bichromate.
- 6.- This reaction is lost partly when the tissues are run up through the alcohols, and entirely in subsequent treatment (clearing oils, heat of the paraffin oven, etc.)

Thus it seems that chromic acid forms here and there in the medullary cells a more or less unstable compound with something contained within the cells. This union may be made more stable, however, and very uniform by formol solution which probably acts in

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the capacity of an accentuator.

In comparing my results with those of Schur and Wiesel,
I have reached the conclusion that the reaction which they describe
as disappearing during anesthesia is identical with that which I
have mentioned above (5 and 6). The readiness with which it disappears is the cause, I believe, of their reaching the conclusion
that it was lost by anesthesia. That the medulla loses its peculiar
affinity for chromic acid under the influence of ether, I am convinced is not the case, and therefore I have felt justified in using
this anesthetic in the experiments.

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#### NORMAL HISTOLOGY OF THE ADRENAL GLANDS

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CAT AND DOG.

It is not the purpose of this paper to give an exhaustive account of the histology of the adrenals. A very thorough study (11) of the glands has been made by Flint. In one point however, his work was incomplete and this was in his description of the so-called "Pfaundler granules", which occur in both the cortex and medulla and feature constitute perhaps the most characteristic histological, of the gland. These granules are small bodies which stain with osmium tetroxide, Soudan III, and Scharlach R., and are soluble in absolute alcohol, ether, chloroform, turpentine or any of the fat solvents. Flint mentioned seeing these granules but gave no description of their definite arrangement.

Pfaundler has gone into greater detail concerning these bodies, but his study was almost entirely from a micro-chemical standpoint and he paid very little attention to the anatomical relations of the granules to other cell structures. Although the granules gave all the fat reactions, he did not believe them to be true fat droplets but failed to give his reasons for this opinion. They are best demonstrated by treating tissues with osmic acid fixing fluids (Flemming, etc.) for twenty four hours. Pfaundler described the granules as lying for the most part in groups near the nucleus, but they might be scattered over the whole cell cytoplasm. They appeared in the tissue spaces outside of the cell as well and might coalesce into larger droplets. This extrusion was due to the gentle pressing of the gland in order to get the granules into the

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blood spaces. In my own work, I used every care in handling the gland and as a result I was unable to note the extruded granules which Pfaundler described. He found that the number of granules varied in different individuals; that they decreased in number with the advancing age of the individual; and that they were related in some way with the nourishment of the animals in as much as he found the greater number in a poorly nourished twelve year old stallion.

Biedl described masses of material in the blood vessels of the adrenal, consisting of clear, bright, yellow granules, closely resembling platelets or broken down erythrocytes.

Whether or not these granules are fat droplets, is an undecided question and up to the present time no satisfactory evidence has been brought out on either side. As for the other histological features of the unstimulated gland, they will be treated in only such detail as seems necessary for a clear understanding of the changes which take place during secretion.

The cortex of the adrenal is made up of three zones characterized by the shape and arrangement of the cells:

Zona glomerulosa: The cells of the zona glomerulosa, as is already well known, are arranged in the dcg adrenal in more or less coiled columns. In form they are long, columnar and regularly arranged, approaching each other by their short diameter, and lying side by side. The oval nuclei are nearly centrally placed but do not form strictly parallel rows. The cytoplasm in the stained preparations shows a heavily reticulated network (Fig.4). This Network, as in the other zones appears to surround small vaculated spaces which correspond, I think, to the granules which normally occur in the gland but which were dissolved out afterwards by the treatment of the tissues. The spaces do not seem so numerous as the

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granules but this difference is only apparent and may be attributed not to a difference in the number of granules as compared with the number of spaces, but to the fact that the granules show when cut in any plane, while the reticulated spaces do not. It may be mentioned here that there are in this zone no large vacuolated spaces corresponding to the transitional forms described farther on.

The only fixing fluids which show the granules are those containing osmic acid such as Flemming's Solution. The granules vary in size from one micron down to almost immeasurable dimensions, but the majority are of the one micron size. I find a fairly definite and regular arrangement of the granules in the cells of the normal unstimulated gland, an arrangement not noticed by either Flint or Pfaundler. (Fig.1.) These granules extend down the sides of the cells in rows parallel with its long axis, thus giving the cells a beaded appearance. There are ordinarily two well defined rows on each side of the nucleus with a few other granules scattered in between. The granules which are interspersed are slightly larger than the others and in places may coalesce, but they never reach the size in the cells of the zona glomerulosa that they do in the cells of the other zones. This general arrangement I find to be constant in the unstimulated gland with two exceptions. In the normal unstimulated glands from two dogs, which I studied, the granules are of different appearance. In these two cases, the granules are present, but very few took the osmic acid stain, the others appearing as round shining globules. Poor nourishment or lack of food are certainly not the cause of this peculiarity since the animals were fed regularly. One of the dogs was experimented upon immediately after feeding while the other one was used twenty-four hours after feeding. The difference is not due to lack of penetration of the

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killing fluid in as much as granules farther within the tissue than these, are stained in the characteristic way, but is owing, I believe to a different physiological state of the granules. The granules very rarely appeared in the blood spaces of the zone although occasionally such a condition was noted.

The glomerular zone of the cat adrenal differes slightly from the dog adrenal in that the zone is not so wide relatively and the cells are not so regular. In the stained preparations, there is not the heavily reticulated network as in the dog adrenal but the ordinary cytoplasmic reticulum take its place. Corresponding to this difference, as might be expected, the granules are absent from the Flemming preparations. Aside from these differences, the characteristics of the zones of the two animals are the same.

Zona fasciculata: This zone in the adrenal of the cat and dog as Flint has already noted, is composed of polyhedral cells arranged in anastamosing columns running at right angles to the capsule. The arrangement of the cells is due to the presence of capillaries separating them into columns about two cells wide. In the iron haemotoxylin sections, the nuclei are stained less deeply than those of the zona glomerulosa and are situated centrally in the cells. The cytoplasm of the cells contains the reticulated network which I find to be much heavier and coarser than that of the glomerular zone. This meshwork radiates out from the nucleus all over the cell and does not occur as Pfaundler described it "stellenweise." The zone contains vacuolated spaces corresponding to the positions of certain bodies which I have termed "transition forms" and which will be described in the next paragraph. These spaces occur not only within the cells, but also in the blood spaces where they have distended the vessel walls and have later been disThe state of the s

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In the Flemming preparations, I found the granules to be more numerous in general in the dog that in the cat adrenal. Moreover they are larger, usually reaching a diameter of two micra. Their position in the cell seems to be without reference to the capillaries and other blood spaces. (Fig.1) They may occur in one part of the cell or in another, or again they may surround the nucleus, completely filling the space between the nucleus and the cell walls, but not with any definite arrangement however, as in the zona glomerulosa.

In addition to the smaller granules, I found here as well as in the zona reticularis the "transitional forms" (Fig.1) previously mentioned. These are large hyaline appearing masses varying in size from that of half a cell width to the width of two fasciculata cells. Under the microscope they show all gradations from nearly colorless, through bluish transition to black. These degrees of coloring may be due, I think, to the extent to which the osmic acid is washed out of the preparations, but what seems to me more probable is that they represent different physiological conditions as evidenced by their different chemical nature in regard to the osmic acid. In every case those which are the largest are faintest in color, while the others become more black as they become smaller. That these masses are due to a coalescing of the smaller granules seems beyond doubt. This is evidenced by the facts that there appear occasionally bodies which are irregular in shape and outline as if two or three other smaller masses were meeting and running one into another; that there is a fairly constant inverse ratio between the size of the granules and their number in the cells, and that

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all stages from the smaller granules to the largest types may be seen. Sometimes they are found in the cells but the larger forms invariably appear in the blood spaces where they distend the capillary walls to a great extent. These transition forms become more numerous towards the zona reticularis.

There are relatively fewer transition forms in the cat adrenal than in the dog adrenal, but this would be expected owing to the relatively fewer number of granules.

Zona reticularis: The zona reticularis of the cat and dog adrenal is made up of polyhedral cells which are slightly smaller than the fasciculata cells. The cells are arranged in small groups which are, according to Flint, surrounded by capillary plexuses. The nuclei are generally centrally placed. The whole cell stains darker than the cells of the other two zones, and the reticular network differs from that of the other two zones in that the strands are fewer and the spaces correspondingly larger. The large vacuoles occur more frequently than in the zona fasciculata and therefore correspond to the greater number of transition forms found in the osmic acid preparations.

The Flemming preparations showed granules in this zone.

Pfaundler was unable to find them here but this was no doubt due to the fact that they were pressed out by his treatment. They are however relatively fewer in number than in the other zones. They may be situated so as entirely to surround the nucleus but more often they are in clumps of three or four, midway between the nucleus and the cell wall. (Fig.1) The transition forms are more numerous and larger than in the zona fasciculata. The coalescing stages are also more numerous.

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As in the other zones these granules and transition farms are fewer in the cat adrenal than in the dog adrenal.

Medulla: The medullary cells show few granules in the osmic preparations. The cytoplasm in the iron haemotoxylin preparations is evenly granular throughout and does not contain the reticular network characteristic of the cortical cells. When granules are present they are small and situated at the outer edge of the cell. Granules appear occasionally in the blood spaces of this region. No coalescing forms are noted here but the transition forms are seen in the blood spaces where they may reach the size of two medullary cells.

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#### HISTOLOGY OF THE STIMULATED ADRENAL GLANDS.

As a result of stimulation certain marked changes occurr in the adrenal gland. (Fig. 3) For the description of these changes, I will describe first, a gland which was stimulated for two hours.

The animal used in this experiment was a dog (NO.7) weighing nineteen kilograms. No cannula was inserted into the adrenal vein but the blood was allowed to flow through uninterruptedly. The left splauchnic nerve was severed after it emerged from the diaphragm and was stimulated at its peripheral end with a weak interrupted current for two hours in half-minute periods alternating with equal periods of rest. I take this as a typestudy in as much as the changes occurring as a result of stimulation are exagerated owing to the fact that the gland was more nearly exhausted.

Zona glomerulosa: The cell walls in this zone are almost entirely obliterated, that is, the dividing lines between the cells are not clearly made out.(Fig.5) In places the cells are plainly divided by the differentiated protoplasm, in other places this is partly gone, while in still other regions no differentiation between the cells is to be noted. This is in marked contrast to the gland of the opposite side which shows perfect cell walls and normal structure throughout. The nuclei are irregularly placed and have lost their parallel arrangement in rows. The reticulated network is broken up and small dissociated masses are scattered unevenly over the cytoplasm of the cells. Such an appearance might be due to one of several causes. It may be taken as an evidence of degeneration, of trauma, or it may be due to the effect of stimulation. That the changes are due to the last cause, however, I feel sure. If degen-

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eration were taking place, the nuclei would be the first to be effected. This does not appear to be the case in as much as the nuclei are of the normal size, are regular in outline, and although some few do stain very deeply, it is a condition which is due I think to an irregularity of staining, for the cells of the opposite unstimulated gland show an equal number of such nuclei. Moreover, if the gland were previously diseased, as for example in Addison's disease, the opposite gland would be also affected pathologically. In this case the right gland is perfectly normal, the cell walls are undisturbed and the nuclei maintain their definite arrangement. That the change is due to trauma also seems improbable. In the treatment of the gland every precaution was taken against rough handling, and if the differences are due to this factor they should show up equally as marked in the opposite gland. From these facts it seems to me that the stimulation is the cause of the differences.

In the Flemming preparations, the granules have partly disappeared so that the cells lose their characteristic beaded appearance. (Fig. 2). The interspersed granules are also fewer in number while more of these bodies appear in the blood spaces, even yet, though not in such numbers as Pfaundler described. In the unstimulated gland of the right side the granules are present in the usual number and arrangement, with the exception that some few in the glomerular cells are unstained with the osmic acid.

Zona fasciculata: The zona fasciculata is the least changed of all the zones as a result of stimulation. The cells are slightly shrunken and in some places the cell walls are gone although there is no definite occurrence of these regions. This however does not occur to the extent that it does in the zona glomerulosa. The nuclei may lie either centrally or at one side of the

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cell. One case seems to occur as frequently as the other. There is little change in the reticulated network of the cytoplasm with the exception that the strands are fewer corresponding to the fewer are larger granules which occur.

The granules in the Flemming preparations are more of the coalescing form than usually appear in the unstimulated gland.

(Fig.2) For this reason they are fewer and relatively larger. The transition forms are absent in the stimulated gland.

The cell cords are pulled apart very often as if the capillaries have been crowded at some time with granules.

Zona reticularis: The greatest change as a result of stimulation occurs in this zone. The cell walls are nearly entirely gone, the reticulated network is broken up and scattered in small masses, while the nuclei as well as the cytoplasm of the cells stains darker than in the other zones. (Fig.6) The contour of the nuclei appear regular.

No transition forms appear after treatment with Flemmings Solution, and the granules are fast coalescing and disappearing.

(Fig.2) A number of granules appear in the blood spaces between the cells.

Medulla: No cellular changes are seen in the medulla as a result of stimulation. The only difference which is to be noted is the fewer number of granules in the cytoplasm of the cells and the increased number in the medullary venous spaces. The transition forms occur more frequently also, than in the unstimulated glands. The chromaffine reaction appeared to be unchanged, the patch of cells becoming neither less in number or losing any of the intensity of the color reaction.

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In the gland, from the cat, which was stimulated for twenty minutes and in which the adrenal artery was occluded, practically the same appearances are seen. In the zona glomerulosa the cell boundaries are indistinct, the nuclei are irregularly placed and do not stain deeply, while the granules are of course entirely absent, this being normally the case in the cat adrenal. Transition forms occur here in this zone in the Flemming preparation, for the first time, but they are few in number.

In the zona fasciculata similar changes take place but not to such a marked degree. The reticulated network is broken up in to small masses. The preparations which were treated with Flemming's Solution show scarcely any granules in the cell cytoplasm, while the transition forms are correspondingly more plentiful especially in the blood spaces.

In the zona fasciculata the granules disappeared almost entirely and the transition forms occurred in greater numbers than in the other two zones.

When the adrenal vein was occluded and the gland stimulated the blood vessels and capillaries were so congested especially in the zona glomerulosa that any granules which might have been given off as a result of this treatment are hidden by erythrocytes.

In the zona fasciculata and zona reticularis, the only difference aside from the congestion is the increased number of transition forms occurring in the blood spaces, there being an enormous number in the last zone.

The medulla likewise shows a number of these bodies in the blood spaces.

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Other glands were stimulated for various periods of time ranging from ten minutes to two hours although most of the experiments were os one-half hour stimulation. In all, six dogs and two cats were used for stimulation. The results obtained in the histological study of these glands are practically the same as in the typical case, the only difference being in the degrees of change corresponding to the various periods of time of stimulation.

After ten minutes stimulation no change appears in the gland.

Marked changes do show however between twenty and thirty minutes stimulation (Fig.7) in that the cell structures, with the exception of the nuclei begin to break down and the granulas to coalesce and disappear.

I was able to obtain the adrenal glands of a dog which had died of starvation. The glands were taken out immediately and pieces were put into Flemming's Solution. A study of these glands shows that the granules have disappeared from the zona glomerulosa. The zona fasciculata appears the same as usual but the zona reticularis contains an increased number of the coalescing forms while the transitional forms are almost entirely absent. A slight increase of granules is noticed in the medulla. This appearance is very much like that of the stimulated gland with the exception of the slight increase of granules in the medullary cells.

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#### DISCUSSION AND SUMMARY.

In summarizing the results of the investigation, I may say that certain distinct and characteristic changes occur as the result of stimulation. Very noticeable cellular changes take place and the granules which appear to be one of the characteristic structures of the gland show a progressive decrease in number in the stimulated gland. Moreover new bodies are formed as the result of coalescing of these granules. The chromaffine reaction which Schur and Wiesel thought was in some way related to the active principle of the gland remains unchanged even after two hours stimulation. These differences between the stimulated and unstimulated gland, are, I believe, a histological verification of Dreyer's work on secretory nerves to the adrenal.

Changes such as do occur might be due to one of several causes, which include trauma, degeneration, blood flow through the gland, or stimulation. The factor of trauma however may be eliminated in as much as the gland remains untouched until it is taken from the body after stimulation. Moreover if this were the case, there would be some histological evidence, in the way of lencocytes appearing in the region of the injury and this does not occur to such an extent as to warrant this explanation. It is very improbable that autolysis would set in during the relatively short spaces of time during stimulation, especially so since the flow of blood through the gland is unimpaired. That the changes are due to the blood flow through the gland also seems improbable. Dreyer found that the active principle was given off as the result of stimulation equally well when the flow of blood through the gland was

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increased, decreased or remained constant. My own work seems to confirm histologically this fact. In the gland, which the adrenal vein was occluded, the congestion of the blood vessels within the gland would suggest considerable pressure. The granules in the capillaries are obscured by reason of so many erythrocytes within the vessels but the cells themselves show no changes except a slight irregularity due to the distension of the blood vessels. granules within the cells appear the same as in other glands stimulated a corresponding length of time. The pressure exerted by this congestion seems to be much more than normally occurs even with the increased blood flow which results from stimulation. Likewise in the case where the adrenal artery was occluded and the blood flow therefore correspondingly out off, the granules disappeared very quickly. This practically climinates such factors as might appear to produce these changes which occur after stimulation and leaves stimulation the cause of the histological differences.

The transition forms in their last stages are, I believe, the real secretion of the gland, although I have not as yet obtained experimental proof fully warranting such a conclusion. The facts which lead to this theory are as follows: The granules as they occur in the cells of the cortex are given off in relatively few numbers as a result of stimulation and even these free granules may be due to the handling of the gland when it is taken from the body, no matter how much care be used in the procedure. For this reason I take the cellular granules to be the prozymogen state of the transition forms. Again, all states of transition are found as well as all stages of coalescence from the most minute granules up to the larger transition forms. The largest discernable tran-

sitional forms sceningly have a different chemical nature from the others in as much as there is a regular gradation in the amount of stain taken up when they are treated with osmic acid fluids. I think that ultimately, i.e. when they are in condition to be used by the body, they are unstainable by the osmic acid and for this reason are not seen in the microscopic study, but again I have not experimental evidence enough to state this as a fact and therefore for the time being I suggest it as a theory. Finally, the largest forms which are to be seen in the study of the tissue invariably appear in the blood spaces and this would suggest the secretion theory.

There seems to be a difference in the activity of the zones. The zona fasciculata appears to act in the capacity of a store house for the granules since the least change after stimulation occurs in this region. On the other hand the zona glomerulosa is active while the zona reticularis is still more so. In fact the greatest activity appears to be in the latter zone. Following stimulation there occur in this zone, notable cell changes, the greatest number of coalescing forms corresponding to the quick disappearance of the small cellular granules and an increased number of transition forms. Especially remarkable is the enormous number of these transition forms which occur when the adrenal vein is occluded. Just what part the zona glomerulosa plays, I am unable to say but that it has some function seems beyond question since marked cell changes as well as a disappearance of the granules occur after stimulation; although the coalescing forms are rare and the transition forms are absent.

It would seem from a study of the histological appearance of the medulla after stimulation that no changes occurred in this

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paratively rare. No cellular changes take place as a result from stimulation and the chromaffine reaction appears to be no different from that found in the unstimulated gland.

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#### FIGURES.

- 1. Unstimulated adrenal Flemming
- 2. Stimulated Adrenal Flemming
- 3. Stimulated Adrenal -- Zenker--Iron Haemotoxylin
- 4. Zona glomerulosa cells unstimulated gland--Zenker--Iron haemotoxylin
- 5. Zona glomerulosa cells--stimulated gland--Zenker--Iron haemotoxylin
- 6. Zona reticularis cells--stimulated gland--Zenker--Iron haemotoxylin
- 7. Zona glomerulosa cells--after 20-30 minutes stimulation-Zenker-Iron haemotoxylin

#### DESCRIPTION OF FIGURES.

- Z.G.- zona glomerulosa
- Z.F. zona fasciculata
- Z.R.- zona veticularis
- M. medulla
- T. -- transition forms
- C. -- coalescing forms
- G. . granules
- V. -- vacuolated spaces
- R. . reticuolated network

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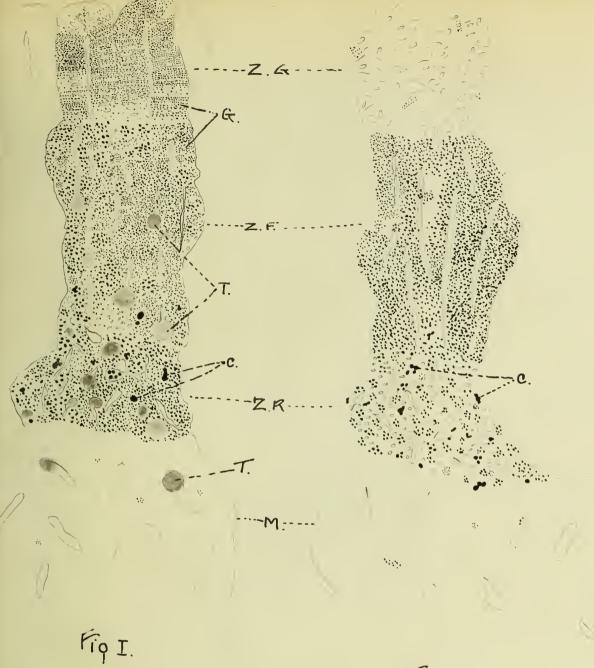
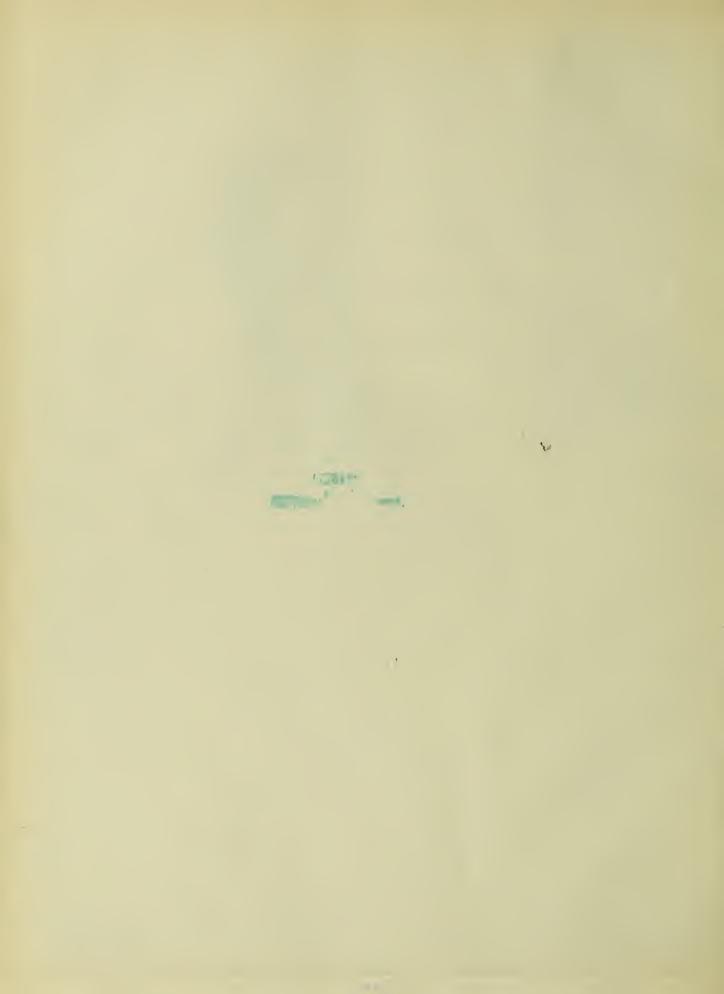


Fig II.



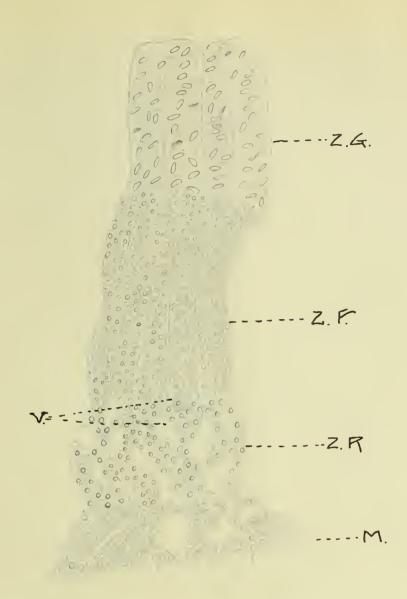


Fig.III







Fig.VI.



Fig.VII.



